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13. ABSTRACT

Oxygen consumption, body temperature, plasma lactic acid and ketones were measured in male mongrel dogs, anesthetized, paralyzed, artificially ventilated and cooled in a water bath at 34° C. Treatment included inspiration of 10 or 20% CO_2, β adrenergic blockade (propranolol), and β blockade plus 10% CO_2. All treatments resulted in a lower oxygen consumption than controls ($P < .05$) with the 20% CO_2 group showing a greater depression than the other three experimental groups ($P < .05$). The rate of decline in body temperature was greater while breathing 20% CO_2 than room air ($P < .05$). β adrenergic blockade resulted in a drop in lactate levels compared with controls ($P < .05$), while a further drop occurred with β blockade plus 10% CO_2 ($P < .025$). Plasma free fatty acids tended to decrease with propranolol or 10% CO_2, but the only significant difference detected was an increased downtrend during the first 30 min with the combined treatment. It was concluded that the increased rate of decline of body temperature with hypercapnia was due to decreased heat production primarily by inhibition of the β-adrenergic calorigenic processes.

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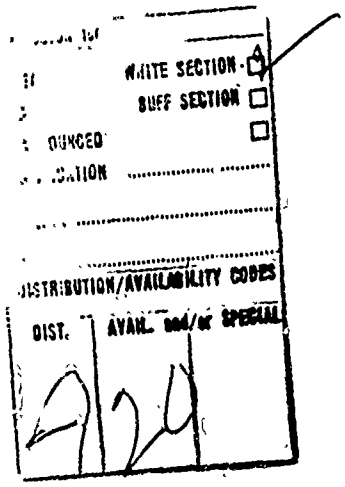

Cooling of Anesthetized Paralyzed Dogs During Hypercapnia and B-adrenergic Blockade

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PEPELKO, W. E., and S. M. CAIN. *Cooling of Anesthetized Paralyzed Dogs During Hypercapnia and β-adrenergic Blockade.* Aerospace Med. 43(3):287-290, 1972.

Oxygen consumption and body temperature were measured in male mongrel dogs, anesthetized, paralyzed, artificially ventilated and cooled in a water bath at 34° C. Treatments included inspiration of 10% or 20% CO_2, β-adrenergic blockade (propranolol) and β-blockade plus 10% CO_2. Oxygen consumption in ml/kg/min STPD after water immersion showed an average increase of 0.41 in air breathing controls but decreased an average of 0.31 with β-blockade, 0.69 with 10% CO_2 inspiration, 0.76 with 10% CO_2 plus β-blockade and 1.35 with 20% CO_2 inspiration. All experimental groups had a lower post-treatment oxygen consumption than controls, ($P < .05$) with the 20% CO_2 group exhibiting a greater depression than the other three experimental groups ($P < .05$). The rate of decline in body temperatures was greater while breathing 20% CO_2 than room air ($P < .05$). It was concluded that hypercapnia inhibits β-adrenergic calorigenic mechanisms while having little apparent effect upon heat loss.

A POTENTIAL HAZARD of closed environments such as spacecraft, submarines or underground locations is a buildup of CO_2 in the gaseous environment. Among the physiological effects of hypercapnia is a decrease in the ability to maintain body temperature. This lessened thermoregulatory capacity has been reported in dogs,[1] humans,[2] rats[21 5] and mice.[10] A decline in body temperature could be due to either an inhibition of calorigenesis, an increase in heat loss, or both. Heat production can be influenced by voluntary movement, the work of breathing, shivering, or a basic change in chemical processes at the subcellular level. Heat loss, on the other hand may be influenced not only by circulatory changes, but also by the degree of piloerection sweating and hyperventilation.

The present experiment was designed to measure changes in body temperature and heat production in anesthetized paralyzed dogs, artificially ventilated and cooled in a water bath at 34°C while breathing gas mixtures high in CO_2. In this preparation all variables affecting heat production or heat loss were eliminated with the exception of changes in circulation and nonshivering thermogenesis. In addition, the effects of hypercapnia were also compared with that of a β-adrenergic blocking agent which influences thermoregulation primarily by inhibition of non-shivering thermogenesis[20].

METHODS

Forty mongrel dogs weighing 11 to 25 kg were selected into five groups of equal number with the same average body weight in each group. They were anesthetized with sodium pentobarbital, 30 mg/kg and catheters were placed in a femoral vein for infusion and a femoral artery for sample collection. Following tracheostomy the dogs were respired with a Harvard Model 607 respiration pump. Muscular paralysis was induced with a priming dose of about 2 mg of succinylcholine intravenously, followed by a constant infusion at .23 mg/min. The respiration pump was set to give an arterial P_{CO_2} between 35 to 40 torr while the dog breathed air, and was not readjusted. Hypercapnia was induced with a gas mixture containing either 10% or 20% CO_2 + 21% O_2 from a 71-cu-ft cylinder fitted with a demand regulator and connected to the intake of the respiration pump. β-adrenergic blockade was attained by intravenous injection of 0.5 mg/kg propranolol every 30 min. Previous experience has shown that this dose level and interval would give complete β-blockade under normal conditions. The dogs were cooled by total immersion in a large water bath which was stirred and maintained at a temperature of 34°C by circulating thermostatically controlled water through a copper coil in the bath.

Five ml of arterial blood were collected anaerobically into a heparinized glass syringe and immediately analyzed for pH, P_{O_2} and P_{CO_2} in an Instrumentation Laboratories blood gas analyzer at a temperature of 37°C. The results were corrected to the body tempera-

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The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences-National Research Council.

ture at the time of sampling. Oxygen consumption, corrected to STPD, was measured by collecting the expired gas into Douglas bags, measuring the volume, and analyzing the oxygen fraction with a Beckman E-2 oxygen analyzer and the carbon dioxide fraction with a Beckman Model LB1 carbon dioxide analyzer. Body temperature was measured by placing a thermistor probe in the esophagus near the level of the heart.

After adjustment of ventilation, about 30 min were allowed to elapse or until body temperatures were stable prior to the start of the experiment. Expired gas was then collected for 30 min while the dogs breathed room air. Gas samples were collected for 10-min periods and analyzed. After the control period, the dogs were placed in the water bath. High CO_2 and/or β-blockade was begun immediately after immersion. Gas samples were collected every 10 min and blood samples taken every 30 min. The five experimental groups had eight animals per group. The five experimental conditions were room air, 10% CO_2, propranolol, propranolol + 10% CO_2, and 20% CO_2.

RESULTS

Arterial pH, P_{O_2}, P_{CO_2} and percent oxygen saturation are shown in Table I. Inspiration of 10% CO_2 resulted in a pH between 7.0 and 7.1. With 20% CO_2, arterial blood pH decreased further—between 6.8 and 6.9. Propranolol treatment alone resulted in a slight increase in pH associated with a slight decrease in P_{CO_2}.

Pretreatment oxygen consumption and body temperature are shown in Table II and the percent change in oxygen consumption in Figure 1. The average change in oxygen consumption from pretreatment levels in ml/kg/min STPD were control +0.41; propranolol +0.31; 10% CO_2 −0.69; propranolol + 10% CO_2 −0.76; and 20% CO_2 −1.35. All the experimental groups showed a lower posttreatment oxygen consumption than controls ($P < .05$), with the 20% CO_2 group showing a greater depression than the other three experimental groups ($P < .05$). Although 10% CO_2 appeared to have a slightly greater depressing effect on oxygen uptake than propranolol, this difference was not significant. The combined treatment also failed to depress oxygen consumption significantly more than 10% CO_2 or propranolol alone.

Body temperature changes are shown in Figure 2. Changes with time were fitted to exponential curves; body temperature $= \delta + \alpha e^{\beta t}$. These parameters are shown in Table III. The 20% CO_2 group differed significantly from controls, having a lower estimated asymptote and a greater rate of temperature decline ($P < .05$). The total decrease in body temperature during the 2-hr treatment period averaged 1.00°C for air-breathing controls, 1.59°C with propranolol, 1.68°C with 10% CO_2, 1.65°C with propranolol + 10% CO_2, and 0.72°C with 20% CO_2. A comparison of these mean declines using Duncan's multiple range test also indicated that the group treated with 20% CO_2 differed from controls at the 5% level, while the other three treatment groups had probability values of about .10 when compared with controls. There was no indication of any synergism between CO_2 and propranolol.

DISCUSSION

Although the 34°C water bath was a very mild cold stress, even the room air breathing dogs were unable to maintain their body temperatures completely. Earlier reports have shown that the non-cold adapted dog can only increase non-shivering thermogensis to a small extent.[6] Our room air breathing dogs were able to increase oxygen consumption an average of only 0.41 ml/kg/min while immersed in the water bath. Since the animals could not shiver, their defense against cold was quite limited. It is possible that the decline in temperature itself may have affected metabolic rate even though this decrement in body temperature was quite small. To test this possibility, oxygen consumption was measured in two dogs exposed to 10% and 20% CO_2 in a thermo-

TABLE I. ARTERIAL BLOOD GAS AND pH MEASUREMENTS OF DOGS BEFORE AND DURING IMMERSION IN A 34° C WATER BATH N = 8 FOR ALL GROUPS.

Time in minutes	−30	0	30	60	90	120
			Arterial pH			
Control	7.352 ± .036*	7.352 ± .026	7.382 ± .018	7.393 ± .035	7.378 ± .039	7.380 ± .046
10% CO_2	7.349 ± .021	7.348 ± .031	7.061 ± .028	7.014 ± .020	7.037 ± .038	7.051 ± .037
Propranolol	7.363 ± .032	7.372 ± .037	7.397 ± .029	7.407 ± .034	7.393 ± .039	7.403 ± .035
10% CO_2 + Propranolol	7.356 ± .020	7.346 ± .028	7.039 ± .028	7.044 ± .032	7.034 ± .036	7.053 ± .040
20% CO_2	7.365 ± .023	7.362 ± .039	6.879 ± .038	6.849 ± .014	6.850 ± .069	6.869 ± .067
			Arterial Blood P_{O_2}			
Control	78.0 ± 2.7	76.2 ± 4.9	70.5 ± 6.5	67.7 ± 7.5	64.7 ± 7.1	63.6 ± 6.4
10% CO_2	79.2 ± 7.1	79.2 ± 7.5	84.1 ± 5.9	81.8 ± 8.8	80.8 ± 6.7	79.6 ± 8.0
Propranolol	78.8 ± 8.6	78.1 ± 7.2	72.3 ± 8.9	75.5 ± 8.3	72.4 ± 9.9	68.8 ± 12.7
10% CO_2 + Propranolol	79.3 ± 7.4	78.6 ± 7.4	83.6 ± 18.6	83.1 ± 15.5	82.4 ± 14.6	82.4 ± 14.3
20% CO_2	82.0 ± 4.2	80.2 ± 7.6	88.9 ± 9.0	86.1 ± 11.0	82.3 ± 12.2	81.1 ± 12.6
			Arterial Blood P_{CO_2}			
Control	36.1 ± 2.3	36.0 ± 3.0	35.4 ± 3.2	35.7 ± 3.4	35.6 ± 3.0	35.3 ± 4.5
10% CO_2	36.5 ± 3.9	36.1 ± 3.0	77.6 ± 8.5	83.8 ± 12.3	86.3 ± 12.2	88.5 ± 10.1
Propranolol	36.4 ± 2.4	36.1 ± 2.6	33.0 ± 2.7	32.0 ± 2.0	31.6 ± 2.5	31.8 ± 2.8
10% CO_2 + Propranolol	38.0 ± 2.3	37.9 ± 4.0	83.6 ± 8.4	91.1 ± 5.4	84.6 ± 6.9	88.5 ± 5.5
20% CO_2	36.8 ± 3.2	38.0 ± 3.1	120.3 ± 5.0	119.6 ± 6.7	121.6 ± 7.2	114.5 ± 11.3

* Standard deviation

neutral environment. The decline in metabolic rate was similar to those placed in the water bath and is in agreement with earlier work.[4,14,22]

A comparison of the metabolic effects of CO_2 and propranolol showed that 10% CO_2 inspiration resulted in the same or a slightly greater decline in oxygen consumption than with propranolol alone. However, when 10% CO_2 and propranolol were given together, the decrease in oxygen consumption was no greater than with 10% CO_2 alone. Since the dosage of propranolol was adjusted to give complete beta adrenergic blockade, 10% CO_2 evidently inhibits the same calorigenic mechanism as propranolol. The acute calorigenic response to cold has been shown to be primarily catecholamine mediated.[9] Moreover, despite its stimulation of catecholamine release,[12,15,17,18,19] hypercapnia also inhibits some of the functional effects of catecholamines.[16] The evidence therefore suggests that CO_2 blocks catecholamine mediated calorigenesis and does so despite an increase in circulating epinephrine and norepinephrine levels.

If it is assumed that 10% CO_2 results in complete blockage of beta adrenergic calorigenic mechanisms, then 20% CO_2 must have an additional effect over and above that due to beta blockage. Even with 10% CO_2 there is a slightly larger decline in metabolism, although nonsignificant in this case, than with propranolol alone. As intracellular hydrogen ion levels increase with increasing P_{CO_2}, many enzymes will be further removed from their pH optimum. It is thus likely that very high levels of CO_2 result in a general inhibition of many enzyme systems including those involved in calorigenesis.

An increased rate of body temperature decline occurred during severe hypercapnia despite the elimination of many responses that can affect thermoregulation, such as sweating, piloerection, hyperventilation, and shivering. The difference between dogs breathing air or CO_2 was small, and of borderline significance except in the group inspiring 20% CO_2. Since, in earlier studies, hypercapnia resulted in a more marked depression of body temperature than in the present experiment,[2,5,11] it is probable that some of the thermoregulatory responses eliminated in this study such as shivering and piloerection are affected by CO_2.

The relatively small decline in body temperature that did occur during hypercapnia or β-blockade can easily be accounted for by a decrease in metabolic rate. Moreover, propranolol has been shown to exert its hypothermic effects primarily by inhibition of nonshivering thermogenesis.[20] Nevertheless, the possibility that CO_2 inhalation results in increased heat loss through peripheral vasodilatation cannot be ruled out. Many reports indicate that the direct effects of CO_2 are vasodilatory.[1,7,13] However, in recent studies, hypercapnia resulted in only small irregular changes in forelimb resistance in

TABLE I. PRETREATMENT OXYGEN UPTAKE AND BODY TEMPERATURE. V_{O_2} WAS CALCULATED FOR EACH DOG FROM 3, 15-MIN SAMPLES, WITH 8 DOGS PER GROUP.

	Room air	Propranolol	10% CO_2	10% CO_2 + Propranolol	20% CO_2
V_{O_2} ml/kg/min STPD	6.08 ± 0.72*	5.69 ± 0.71	6.33 ± 0.88	5.88 ± 1.33	6.58 ± 1.01
Body temperature °C at 0 time	37.5 ± 0.68	37.5 ± 1.05	37.5 ± 0.80	37.8 ± 0.63	37.4 ± 0.74

*Standard deviation

TABLE II. PARAMETER ESTIMATES OBTAINED FROM FITTING BODY TEMPERATURE MEANS TO THE EQUATION BODY TEMPERATURE (°C) = $\gamma + \alpha e^{-\beta t}$. N = 8 DOGS PER GROUP WITH 13 MEASUREMENTS PER DOG.

Group	γ	α	β
Control	36.3	1.41	.016
10% CO_2	35.2	2.47	.011
Propranolol	35.6	1.96	.015
10% CO_2 + Propranolol	35.2	2.18*	.012
20% CO_2	31.3*	3.80*	.013

*Significantly different from controls ($P < .05$)

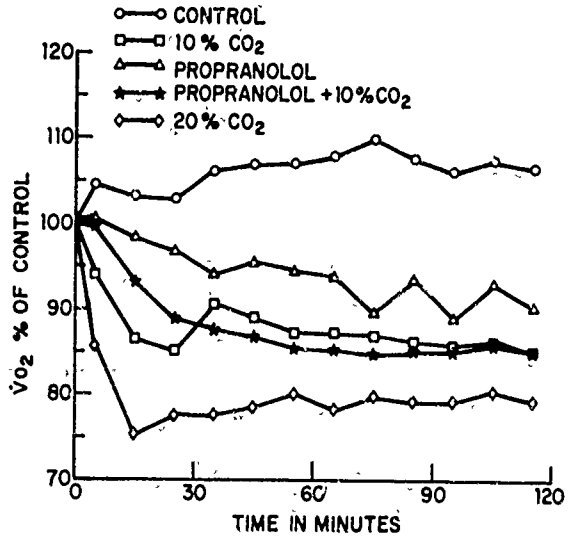

Fig. 1. The percent change in oxygen consumption during 120 min of exposure to the experimental environments and immersion in a 34° C water bath. All groups showed a significant drop in V_{O_2} compared with controls ($P < .05$), with the 20% CO_2 group showing a greater drop than the other three experimental groups ($P < .05$).

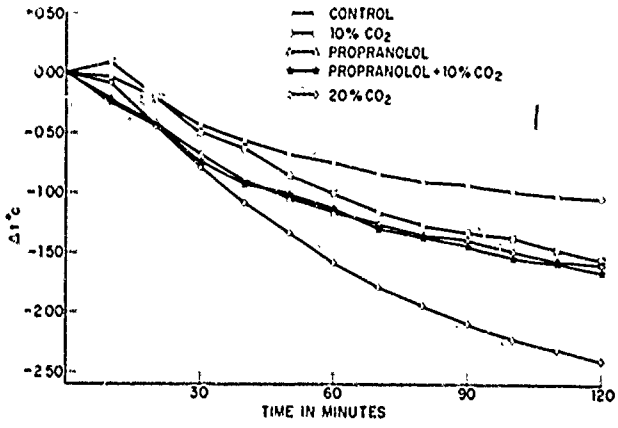

Fig. 2. The decline in body temperature during 120 min of exposure to the experimental environments and immersion in a 34° C water bath. Exposure to 20% CO_2 resulted in a more rapid drop in body temperature than control ($P < .05$).

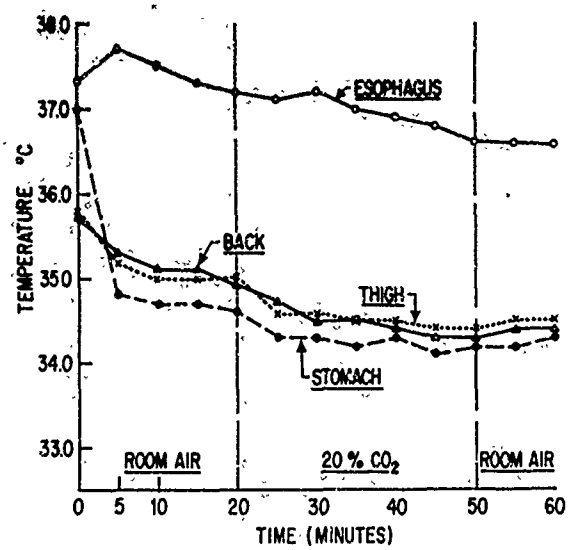

Fig. 3. The effect of 20% CO_2 upon skin and esophageal temperature of a dog during immersion in a 34° C water bath.

intact animals[8] even though the same workers found that resistance decreased markedly in the isolated forelimb adjusted to similar arterial[7] P_{CO_2}. These authors concluded that the locally vasoactive effects of hypercapnia are effectively antagonized by remote actions. Skin and rectal temperature measurements of dogs and humans during hypercapnia also gave no indication of a shift of blood flow to the periphery.[11,13] CO_2 levels, in these two studies however, were lower than used in the present experiment.

We attempted to evaluate the peripheral vasomotor effects of CO_2 by measuring skin temperatures of 5 additional dogs exposed to 10% or 20% CO_2. Initial measurements were made in thermoneutral environments followed by cooling either by blowing cold air on them or by immersion in a 34°C water bath. Skin temperature measurements were made during water immersion by placing thermistors, sensor side uppermost, under the skin and suturing the openings; otherwise, the thermistors were placed on the surface of the skin. CO_2 inspiration resulted in either no change or a small increase in the degree of vasoconstriction when the dogs were cooled. This could be seen by a small decline in skin temperature which often occurred at the onset of CO_2 inspiration coupled with 0.1 to 0.2°C rise in esophageal temperature during the first few minutes of exposure. A sudden rise in core temperature coupled with declining heat production must be due to a decrease in blood flow in the periphery. Figure 3 shows a typical example of a dog cooled in a water bath and breathing 20% CO_2. The temporary rise in esophageal temperature is also seen in Figure 2 for dogs breathing 10% CO_2. Whether vasoconstriction actually occurred with CO_2 inspiration could not be definitely stated with such qualitative measurements. In any case, there was no evidence of increased blood flow to the skin, at least during the initial exposure to hypercapnia. Further work should be conducted not only to quantitate the effects of hypercapnia upon circulatory heat loss but also to determine if the amount of heat loss varies with time of exposure.

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